33* Sodium 4-phenylbutyrate induces IL-8 expression in CF lung epithelial cells through an ERK1/2-dependent pathway

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Sodium 4-phenylbutyrate (4-PBA), a butyrate analogue that is approved for clinical use in cystic fibrosis (CF) lung disease, has been shown to correct the ΔF508-CFTR trafficking defect, to restore CFTR function at the plasma membrane of cultured CF lung epithelial cells and to cause a improvement in nasal epithelial chloride transport in ΔF508-homozygous cystic fibrosis patients. The aim of our study was to gain insights into the potential effects of 4-PBA on the inflammatory response in CF lung epithelial cells. With two CF bronchial epithelial cell types (CFBE14O- and IB3–1 cell lines with ΔF508-homozygous and heterozygous genotype, respectively), we clearly demonstrated that 4-PBA induced a strong increase of both IL-8 mRNA and protein expression in two CF cell lines whereas no significant variation of IL-1β mRNA was observed. Unexpectedly, we also reported that treatment of IB3–1 cells with 4-PBA alone (10 mM) or in combination with 10 ng/ml TNF-α did not increase the NF-κB transcriptional activity and reduced the 20S proteasome activity. These data prompted us to investigate other potential pathways controlling the IL-8 expression by 4-PBA. Inhibition of ERK1/2 signalling pathway by U-0126 blocked the increase of 4-PBA mediated IL-8 expression, suggesting that ERK regulated 4-PBA mediated IL-8 expression in an NF-κB-independent manner. Thus, a combination of 4-PBA treatment with an ERK inhibitor may be beneficial to reduce the lung inflammation in CF patients.

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34* ERK1/2 activation and IL-8 secretion of Cystic Fibrosis lung epithelial cells in response to oxidative stress

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Dysregulated IL-8 production is reported in lung epithelium of cystic fibrosis (CF) patients. We here document the impact of cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction on the production of interleukin (IL)-8, expression of its CXCR1/2 receptors and the involvement of MAP kinase and NF-κB pathways in cystic fibrosis (IB3–1) and (S9) corrected lung epithelial cell lines in response to oxidative stress. We show that expression of both IL-8 and CXCR1/2 receptors is increased in both IB3–1 and S9 cells in response to oxidative stress. Activation of p38 and JNK MAP kinase is similar in the two IB3–1 and S9 cells after oxidative stress. Surprisingly, in contrast to that observed in S9 cells, this is accompanied with no NF-κB activation but a marked ERK1/2 MAP kinase activation is observed in IB3–1 cells. Using a series of chemical inhibitors, we show that the strongest reduction in both IL-8 and CXCR1/2 expression is obtained by treatment of S9 cells with NF-κB inhibitor BAY 11–17082 and with ERK1/2 inhibitor UO126 for IB3–1 cells. Our results point out a switch from NF-κB to ERK1/2 signaling in IB3–1 cells for the regulation of both IL-8 production and CXCR1/2 expression upon oxidative stress.

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35* Presence of β3-adrenoceptor in human lung: overexpression in Cystic Fibrosis

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The β-adrenergic stimulation exerts a variety of effects on airway epithelial cells: increased ciliary beat frequency, opening of apical ion channels such as Cystic Fibrosis Transmembrane conductance Regulator (CFTR). Three β-adrenoceptors (β-AR) subtypes could be involved. β1-AR and β2-AR activate the cAMP pathway, however they are desensitized under a chronic agonist exposure. In a heterologous expression system, we have shown that β3-AR activates CFTR chloride activity independently of cAMP (Leblais et al., 1999, J Biol Chem), but through a Gi/PI3Kinase-MAPKinase pathway (Robay et al., 2005, Mol Pharmacol). As no study reports the presence of β3-AR in the human lung, the aim of this work was to determine its presence in human lung samples and its expression level in CF vs. non-CF patients. Human bronchial samples used for controls were obtained from non-CF patients who underwent a partial lung resection. CF tissues were obtained after a lung graft. β3-AR mRNAs and β3-AR proteins were assayed by PCR and western-blotting respectively. Immunostainings allowed to determine the histological localisation of β3-AR. CF and non-CF β3-AR expression levels were investigated by real-time PCR. By PCR and western-blotting, we detected in all control (n = 4) and CF (n = 3) samples the presence of β3-AR. This receptor was mainly immunolocalised in ciliated epithelial cells. By real-time PCR, we observed a 3–4 fold-overexpression of β3-AR mRNA in CF samples. This study demonstrates for the first time a β3-AR expression in human lung epithelia. Its overexpression in CF suggests a role of β3-AR in this pathology and opens new prospects for therapeutic investigations through β3-AR modulation.

36* A long-acting β2-adrenergic receptor agonist increases hydration and mucus exocytosis in human tracheal glandular cells

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The regulation of CFTR activity is under the activation of surface G protein coupled receptors (GPCR) such as β_2-adrenergic receptor. Many compounds such as the long-acting β_2-agonist (LABA) salmeterol have been shown to activate CFTR through activation of GPCR, which increases the forskolin activated-chloride efflux in the CF-KM4 cells (Castillon *et al*, 2004). We have earlier shown that CFTR protein expression was increased by exposure to the LABA salmeterol (Taouil *et al*, 2003). In addition, we demonstrated an increased ion content and decreased hydration of mucus inside the secretory granules in parallel with a deficient secretory granule expansion through a CFTR-dependent mechanism (Baconnais, Delavoie *et al*, 2005). In this study, we further explored the effect of the LABA salmeterol on the hydration by quantitative dark field intensity, the ion composition by X-ray microanalysis and the expansion of the secretory granules by phase-contrast videomicroscopy in a CF human tracheal glandular cell line (CF-KM4). X-ray microanalysis showed that compared to control, the LABA salmeterol induces in secretory granules a significant ($P < 0.01$) decrease of Na, Cl, P and S concentration and a significant increase ($P < 0.001$) of the hydration. In parallel, we observed that the LABA salmeterol induces enhancement of mucus exocytosis. The incubation of the salmeterol-treated CF cells with a CFTR inhibitor (CFTRinh-172) induced a lower mucus exocytosis. These results suggest that through a CFTR-dependent mechanism, the LABA salmeterol is able to improve the hydration of mucus and therefore may facilitate the process of mucus exocytosis in CF.

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